



Temperature induced shifts in leaf water relations and growth efficiency indicate climate change may limit aspen growth in the Colorado Rockies

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ABSTRACT

Higher temperatures and evaporative demand forecasted for Colorado forests by the end of the century suggest that soil water limitation increasingly will negatively impact whole plant performance. At the same time, upslope or poleward migration of plant ranges in response to warming may result in species experiencing cooler overnight temperatures, particularly if extremes in climate increase. In 2014 we established three experimental gardens along a temperature/elevation gradient to test the implications of shifting temperatures on tree function. Quaking aspen (*Populus tremuloides*) seedlings from a mid-elevation population were established in all three gardens and leaves were sampled monthly during the 2017 growing season. From these, we quantified the magnitude and timing of osmotic regulation, the relationships between leaf osmotic potential (Ψ_{osm}), midday leaf water potential (Ψ_{mid}), and soil moisture conditions under different temperature regimes, and growth efficiency (change in basal area/growing day). We observed a strong relationship between Ψ_{osm} and soil moisture, and a strong seasonal decline in Ψ_{osm} at the warmest and intermediate sites while the coldest site experienced a later increase in osmolytes associated with the highest degree of freeze tolerance. Growth efficiency was highest at the intermediate-temperature site – closest in elevation to the seed source location – but declined asymmetrically with warming or cooling. The novel abiotic conditions at both non-local sites resulted in declines in growth efficiency, suggesting that aspen will experience suboptimal conditions whether it stays in areas experiencing warming temperatures or if it migrates upslope to areas with colder overnight temperatures.

1. Introduction

The plant communities of Rocky Mountain montane forests are being restructured by rising temperatures. In Colorado's mountains, warming has increased $\sim 0.4^\circ\text{C}$ per decade over the second half of the 20th century (McGuire et al., 2012) and is expected to warm an additional 3°C by the end of the 21st century (Gross et al., 2016). Rising temperatures effect trees directly (e.g. increased enzyme activity) and indirectly by altering their water balance via greater evaporative demand, leading to increased desiccation. While warming temperatures in isolation may improve plant performance in some instances, warmer conditions that contribute to water stress typically reduce tree growth (Wu et al., 2011), in part due to decreased photosynthesis as a direct product of reduced stomatal conductance. This is particularly true of systems in the western U.S. where water budgets are already limited due to higher evaporative demand from rapidly warming temperatures and generally low precipitation during the growing season. Thus, even

if precipitation patterns remain unchanged, plants in the future will experience reduced water availability in the soil under warming temperatures alone.

Quaking aspen (*Populus tremuloides*) is a dominant tree in Rocky Mountain forests. Due to a suite of factors (including severe drought, warming temperatures, and pests and pathogens; Worrall et al., 2010; Michaelian et al., 2011), this species has recently experienced substantial population declines – referred to as Sudden Aspen Decline (SAD) – across extensive portions of its range in both montane (Dudley et al., 2015) and subalpine forests (Bretfeld et al., 2016). Deciduous angiosperms in semi-arid, mountainous regions often face a particular challenge; avoiding cellular damage from desiccation during the summer growing season while photosynthesizing sufficiently before the onset of frost and leaf senescence in fall. As soil moisture declines, plants can respond by closing stomata to maintain a constant leaf water potential (Ψ_{leaf}) and hydration of cells, but at the cost of reduced carbon uptake and growth, or plants can maintain positive growth

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while Ψ_{leaf} values decline and endanger the tissues by dehydration. Below water potentials of -1.5 MPa, stomatal conductance, leaf hydraulic conductance, and photosynthetic rate are adversely affected in many species (Boyer, 1970; Bartlett et al., 2016), and if sustained, this eventually leads to mortality via carbon starvation and/or desiccation (Parker and Patton, 1975; McDowell et al., 2008). One way to avoid dehydration in the leaf while continuing photosynthesis is to increase the solute concentration of cells (osmotic adjustment), thus allowing cells to maintain positive turgor as Ψ_{leaf} declines. Seasonal osmotic adjustment (becoming increasingly negative in the fall and winter) has also been correlated with increased frost tolerance (Pramsohler and Neuner, 2013), as additional solutes can reduce the freezing temperature of symplastic water and extend the growing season (see Wisniewski et al., 2003; Webster and Ebdon, 2005). Decreasing soil moisture availability expected with warming temperatures may further impact aspen unless it is able to acclimate and adapt to these new conditions. Hence, quantifying *in situ* seasonal patterns of aspen Ψ_{leaf} and osmotic adjustment under varying soil moisture and air temperature is required to predict how this species will respond to future environmental conditions.

Recent efforts to include plant hydraulic traits in landscape level projections of tree performance highlight the value of this approach in improving estimates of plant mortality (Anderegg et al., 2015), particularly for aspen which appears to primarily rely on shallow soil moisture (< 10 cm, Anderegg et al., 2013). As most trees – and angiosperms in particular – operate with limited hydraulic safety margins (Choat et al., 2012), investigating these traits at xeric local range-limits have proven to be highly predictive of drought-induced mortality (Tai et al., 2017). Yet, we continue to lack information directly linking leaf-level hydraulic stress with overall whole-plant performance (Zwieniecki and Secchi, 2015).

To address these issues, we established three experimental gardens (hereafter sites) that span 6°C mean annual temperature (MAT) but with similar levels of precipitation. Using seedlings across these sites, we investigated the role of warming temperatures on *in situ* tree seedling responses to decreased available soil moisture. We hypothesized that (1) plants growing at the coldest and warmest sites will experience the largest amount of osmotic adjustment in response to cold temperatures (at the coldest site) or reduced soil moisture (at the warmest site). As osmotic adjustment can enhance both drought and freezing tolerance, we expect (2) seedlings in these two sites to exhibit the greatest resistance to frost damage. Finally, osmotic adjustment requires investment of resources by the plant and may alter the growth efficiencies of plants, and so we hypothesize that (3) plants growing at the intermediate-temperature site will have the highest growth efficiency due to a reduction in resources needed for osmotic adjustments.

2. Methods

2.1. Field site

From June through September 2017, measurements were conducted on 5-year-old aspen seedlings planted in three experimental sites established in 2014 and located along a 1200 m elevation gradient on the east-facing slopes of the Front Range of Colorado. Seedling diameters at 5 cm above the ground ranged from 6.1 to 30.3 mm and height from base to terminal bud ranged from 57 to 188 cm. Each site is separated by approximately 3°C MAT but have similar precipitation patterns (~ 475 mm yr^{-1} , see Carroll et al., 2017 for full site description). Seedlings were germinated in a greenhouse from seed collected in a single stand in the Roosevelt National Forest and outplanted when 1 year-old. To standardize site conditions, we selected a flat area in each site with full-sun exposure and removed all vegetation, and we excavated the top 30 cm of soil from two 18.5 m^2 plots in all three sites and filled these areas with soil collected from the intermediate site. We placed a 2m-high fence around each site in 2015 to limit herbivory.

Table 1

Climate and soil data for the experimental sites. Long-term means were derived from 800-m resolution PRISM climate data (PRISM Climate Group, 2004) based on 1986–2011. Experimental data (denoted by *) were calculated based on a time period from 6/1/17 through 9/31/17.

Site	Coldest	Intermediate	Warmest
Elevation (m)	2750	2470	1567
Long term mean annual air temperature ($^{\circ}\text{C}$)	3.7	6.6	9.6
Long term mean annual precipitation (mm)	447	567	409
Experimental* mean daily air temperature ($^{\circ}\text{C}$)	12.5	16.3	19.2
Experimental* min daily air temperature ($^{\circ}\text{C}$)	2.9	10.9	9.4
Experimental* precipitation (mm)	170.6	127.4	137.2
Experimental* mean soil moisture (%)	16.3	12.3	10.8
Experimental* mean soil temperature ($^{\circ}\text{C}$)	13.9	18.1	22.5

Environmental conditions have been continually monitored since 2015 including air temperature and precipitation (Onset HOBO RG3-M), and soil temperature and moisture (Decagon 5 TM Soil Moisture and Temperature Sensor) (Table 1).

2.2. Leaf water potentials

Leaves were excised monthly from south-facing fully-exposed mid-canopy stems on sunny days. Sampling began at 11 h and continued no later than 13 h. One leaf from 7–14 individuals was randomly selected and immediately placed in a Scholander-style pressure chamber (Model 1000, PMS Instrument Co., Albany, OR, USA) to measure midday leaf water potential (Ψ_{mid}). Leaves were then suspended in water and transported back to the lab and allowed to rehydrate for ~ 24 h before being frozen and stored in a freezer (-10°C) for later analysis of osmotic potential (Ψ_{osm}). Frozen leaves were used to estimate Ψ_{osm} by cutting 6 mm leaf discs from leaf lamina tissue, being sure to avoid the mid-vein. Leaf disc initial weight was measured and then leaves were placed in an osmometer (model 5520, Vapro Co. Vapor Pressure Chamber, Wescor, Inc. Logan, UT, USA). The Ψ_{osm} of the leaf disc was repeatedly measured, without opening the chamber, until two readings differed by < 0.01 MPa. Ψ_{mid} values were regressed with volumetric water content (VWC) values collected during sampling and compared among sites. It was determined that the sites had a common relationship and thus were combined to generate an overall relationship. Daily values of midday soil moisture at each site were then compiled and used to predict Ψ_{mid} throughout the growing season at each site. Turgor pressure was also calculated as the difference between Ψ_{mid} and Ψ_{osm} assuming differences in gravitational and pressure potentials were negligible among seedlings.

2.3. Electrolyte leakage

During the final sampling period in early September, pairs of leaves were randomly sampled from five individuals at each site, sealed in separate plastic bags containing a damp paper towel and returned to the lab in a cooler. One leaf of each pair was refrigerated overnight (at 2.77°C) while the other leaf was placed in a freezer (at -10°C) to simulate a nightly freezing event. Leaves were frozen for approximately 18 h followed by two hours in the refrigerator (at 2.77°C) to reach similar temperatures as the samples stored in the refrigerator. Both sets of leaves were then allowed to warm to room temperature for approximately two hours. After warming, leaves were individually submerged in ~ 40 ml of deionized water and conductance was determined (μS , Accumet Model 50 pH/conductivity meter, Fisher Scientific, Pittsburgh, PA, USA.). After the initial conductance was measured, samples were remeasured after: 15, 30, 45, 60, 180, and 360 min to detect maximum electrolyte leakage. Samples were then autoclaved (Steris Steam Sterilizer Model 20VS) at 121°C for 20 min to induce complete cell lysis and final conductance was determined (Sánchez Viveros et al., 2010).

Finally, electrolyte leakage was calculated for each time period as:

$$\text{Electrolyte Leakage (EL)} = \left(\frac{(S_t - S_0)}{S_F} \right) * 100 \quad (1)$$

Where electrolyte leakage equals the change from initial conductance (S_0) to conductance at a time point (S_t), scaled by the final conductance (S_F) and multiplied by 100.

2.4. Growth implications

To quantify the efficiency of growth at each site, the relative change in basal area was determined from the beginning of the growing season (April) to the end of the season (September). Additionally, initiation of bud-burst was tracked at each site in spring 2017 to estimate growing season length (see Carroll et al., 2017 for methods). Percent change in basal area was then divided by the number of growing days at each site. Basal area was calculated using estimates of diameter at 5 cm above the root collar using microcalipers. Stems were marked with paint at 5 cm to ensure accurate measurements each month.

2.5. Statistical analyses

Linear mixed-effects models comparing electrolyte leakage over time were performed, with time as a fixed effect, individual as a random effect, and site as a fixed effect in the full model. Pairwise comparisons were calculated when appropriate using Tukey's HSD ($\alpha = 0.05$). All other relationships were determined in a likelihood framework using simulated annealing global parameter estimation for curve fit optimization. AICc model comparison was used to determine the most parsimonious models in all cases. All statistics were calculated using R (3.3.3) and the 'likelihood' (Murphy, 2015), 'dplyr' (Wickham et al., 2017), 'lme4' (Bates et al., 2015), and 'lsmeans' (Lenth, 2016) packages.

3. Results

3.1. Leaf water potentials

Monthly assessments of Ψ_{mid} revealed that beginning in July seedlings at the warmest site experienced the most negative water potentials while seedlings at the coldest site experienced the least negative water potentials (Fig. 1a). We sampled Ψ_{mid} across the entire range of soil moisture experienced at each site over the study period and found a strong nonlinear relationship ($R^2 = 0.83$ based on AICc model comparison; see Table S1 for ΔAICc weights) across the sites between Ψ_{mid} and VWC (Fig. 1b; 1b inset).

We also observed a strong seasonal effect on Ψ_{osm} with values becoming more negative throughout the growing season (Fig. 2a), although differences in absolute osmotic adjustment were minimal

among the three sites (Fig. 2a inset). Individuals at the coldest, intermediate-temperature, and warmest sites reduced Ψ_{osm} by 1.95, 1.88, and 2.1 MPa respectively. The intermediate and warmest sites experienced increasingly negative Ψ_{osm} between June and July and then maintained roughly constant values throughout the remainder of the experiment. In contrast, individuals at the coldest site experienced increasingly negative Ψ_{osm} each month, with the lowest values occurring in June and the highest values recorded in August. As expected, Ψ_{osm} and VWC were also highly correlated (Fig. 2b). Overall, there was strong evidence of Ψ_{osm} becoming more negative with declining soil moisture ($R^2 = 0.69$, Table S2). These trends suggest that shifts in Ψ_{osm} are driven by seasonal effects (Fig. 2a), and a tightly coupled influence of water availability (Fig. 2b). We assessed the relationship between Ψ_{osm} and soil and air minimum temperatures as well, but there were no significant relationships (Fig. S1). Turgor pressure increased seasonally, peaking earliest at the intermediate-temperature site (closest to the native seed-source location) (Fig. 3); however, initial (June) and final (September) values were similar across all sites.

3.2. Frost tolerance

To investigate the relationship between osmotic regulation and frost tolerance we tested the stability of cell membranes to freezing and the resultant loss of electrolytes from the symplast. AICc model comparison of linear mixed-effects models comparing the effect of including site found a substantial improvement over a model without site ($\Delta\text{AICc} = 55$). Additionally, plants from all sites differed in the amount of electrolyte leakage in response to a simulated freezing event (Tukey HSD for all pairwise comparisons $p\text{-value} < 0.001$; Fig. S2), and as expected the coldest site experienced the least damage due to freezing (the least electrolyte leakage). The intermediate-temperature site experienced the most damage via freezing over the 6 h with the warmest site experiencing moderate leakage relative to the other sites. Additionally, daily minimum temperatures – which were generally highest at the intermediate site, perhaps due to cold air drainage lowering overnight temperatures in the lowest site – throughout the study period were found to reflect the same trend as electrolyte leakage (Fig. S3). These factors resulted in the plants from the intermediate site being the most susceptible to freezing-induced membrane damage and thus the greatest electrolyte leakage among the three sites.

3.3. Growth implications

We predicted that a tradeoff may exist between how efficiently a tree grows and how resources are allocated to leaf structural tissues, osmotic regulation, or frost hardiness. We examined the efficiency of above-ground growth at the different sites by calculating the percent change in basal area per growing day in 2017 (Table 2). The intermediate-temperature site experienced the fastest growth rate based on

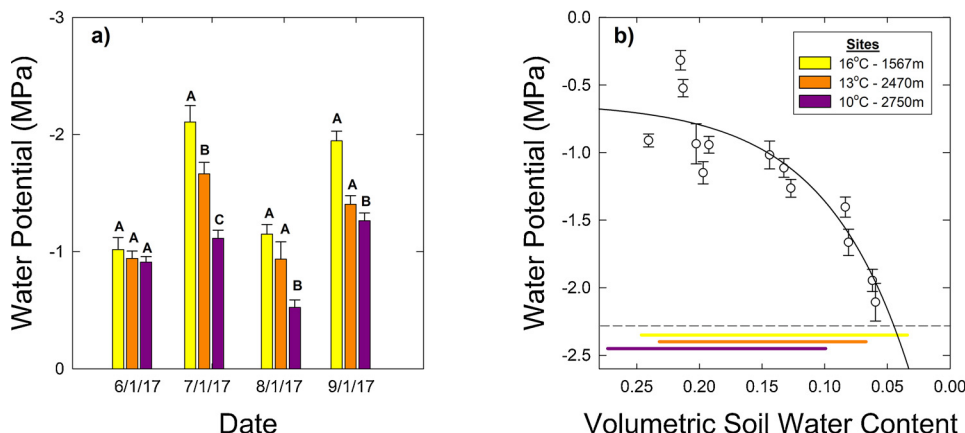


Fig. 1. a) Average midday leaf water potential (Ψ_{mid}) for *P. tremuloides* seedlings throughout the 2017 growing season, bars show 1 standard error and letters denote differences based on Tukey HSD pairwise comparisons. b) Average midday leaf water potential (Ψ_{mid}) relationship with volumetric soil water content (VWC). The modeled relationship shown the curved line is the most parsimonious model based on AICc comparisons ($f = -0.06265936 - 3.207147e^{-14.75403 \times x}$, $R^2 = 0.83$) Horizontal bars at the bottom show the entire range of experimental VWC values observed during the experiment at each site; each color corresponds to the mean growing season temperature of that site.

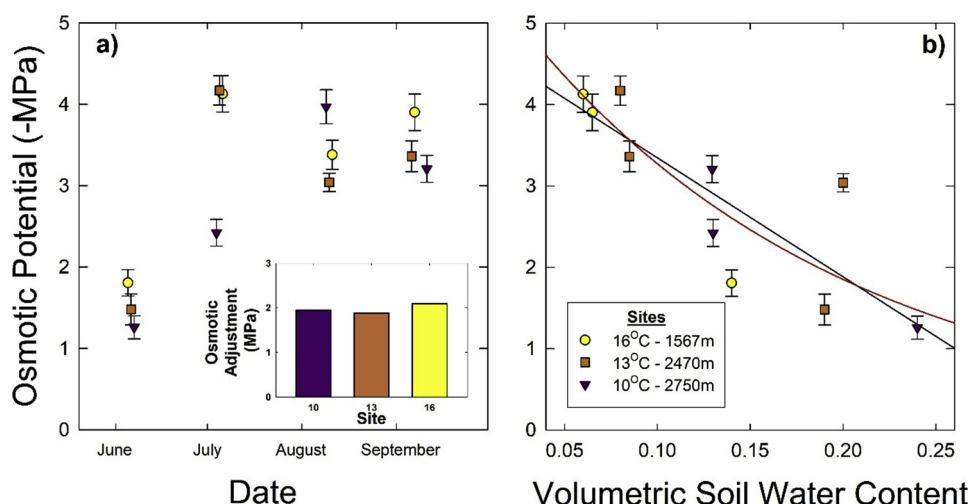


Fig. 2. a) Osmotic potential (Ψ_{osc}) of *P. tremuloides* at each site by sample month. a inset) Site-averaged osmotic adjustment between June and September for each site. b) Ψ_{osc} and soil volumetric water content (VWC). Bars indicate 1 standard error. Linear ($\Delta\text{AICc} = 0$, $f = 4.8069 - 14.6294 * x$, $R^2 = 0.69$) and exponential curves (red, $f = 5.7835 * e^{-5.7004 * x}$, $R^2 = 0.72$) are shown (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article).

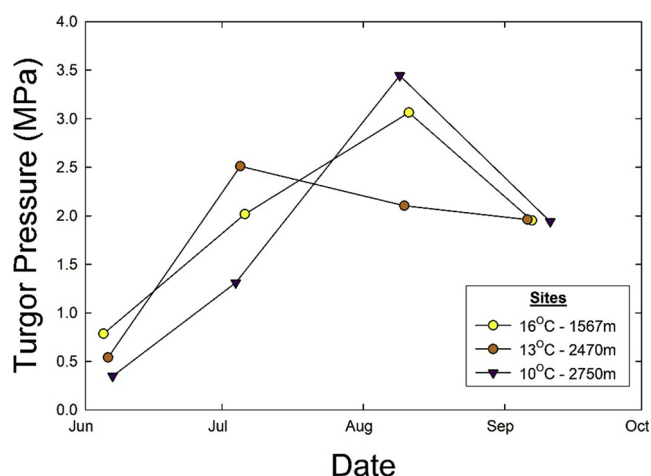


Fig. 3. Average turgor pressure (MPa) for *P. tremuloides* for each site throughout the growing season. Colors correspond to the average growing season temperature of that site.

Table 2

Percent electrolyte leakage from frozen *P. tremuloides* leaves after a period of 6 h versus growth efficiency (percent change in basal area per growing day) of the seedlings at each site throughout the growing season. Values in parentheses are standard errors.

Site (Elevation m)	Electrolyte leakage (SE)	Growth Efficiency (SE)
Warmest (1567 m)	29.39 (3.09)	0.065 (0.022)
Intermediate (2470 m)	45.56 (3.85)	0.278 (0.042)
Coldest (2750 m)	16.11 (6.80)	0.162 (0.021)

Tukey HSD pairwise comparisons, indicating the most efficient growth ($F = 13.47$, $p\text{-value} < 0.001$), followed by the coldest site, and finally the warmest site. Thus, the intermediate-temperature site experienced the greatest leakage and the highest growth efficiency, while the other two sites experienced less electrolyte leakage but declines in growth efficiency compared to the intermediate-temperature site.

Lastly, we quantified differences in the main abiotic limitations: cold temperatures at the coldest/high-elevation site vs. high evaporative demand at the warmest/low-elevation site by quantifying the relationship between Ψ_{mid} and VWC ($R^2 = 0.83$; Table S1). Seedlings at

the coldest site experienced more than a three-fold increase in the number of days where the minimum temperature dropped below zero (Fig. 4a). In contrast, individuals at the coldest site never experienced any days where Ψ_{mid} was more negative than -1.5 MPa, whereas seedlings at the warmest site experienced Ψ_{mid} more negative than -1.5 MPa around 50% of the time (Fig. 4b). Individuals at the intermediate-temperature site experienced the most even distribution of Ψ_{mid} with the lowest number of days in between -1 and -1.5 MPa. These differences in Ψ_{mid} occurred despite similar amounts of precipitation from June through September 2017 at all three sites, suggesting that the effect of warmer temperatures on evaporative demand was an important driver on plant water relations.

4. Discussion

Large areas of aspen forests in the western USA are expected to become increasingly unsuitable climatically over the next 70 years (Rehfeldt et al., 2009), leaving many of its populations in suboptimal conditions. With future warming, aspen populations will respond in two basic ways: with a shift in distribution upslope and poleward via dispersal and regeneration of new individuals, and for extant trees, by adapting to warming in their current environment. Both responses necessitate trait plasticity to be successful. Extant populations will experience increases in MAT, evaporative stress, and more variable soil moisture conditions, while populations growing upslope are likely to experience increased climate extremes (freezing conditions). Hence, we need to consider the effects of cold temperatures as well as warming and increased evaporative demand on aspen hydraulic performance.

When faced with decreasing available soil moisture, aspen allows leaf water potential to decline with soil moisture (Fig. 1b), yet they maintained turgor by adjusting osmotically as indicated by the relationship between soil volumetric water content and osmotic potential (Fig. 2b). By maintaining turgor under limiting soil moisture, plants are able to minimally continue photosynthesis, although this effect is often transient (Puigdefabregas and Pugnaire, 1999). Seedlings at the warmest and intermediate-temperature sites decreased Ψ_{osc} earlier in the year (July) than those at the coldest site likely responding to water limitation. The coldest site which did not experience water limitation due to cooler temperatures, adjusted later in the season. Thus the timing of this is highly sensitive to the onset of abiotic stress at each site. Still, it was surprising that the amount of overall seasonal osmotic adjustment was similar (~2 MPa) at each site (Fig. 2a-inset) given the

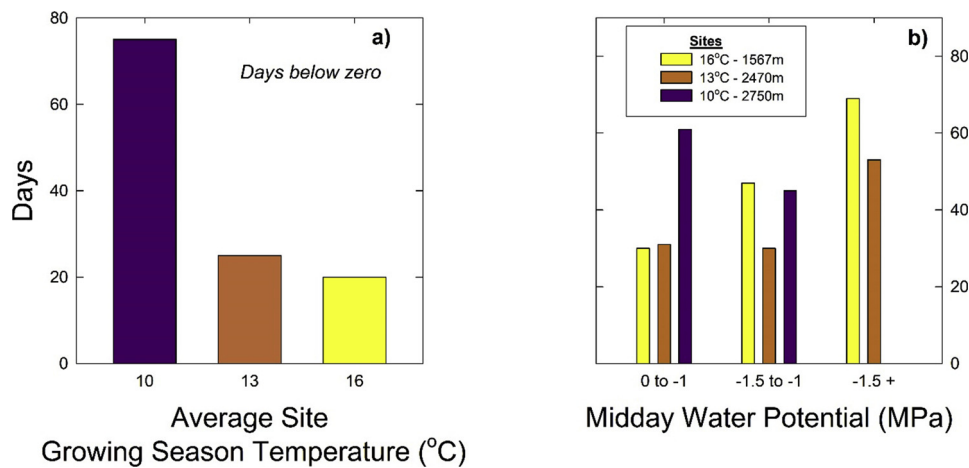


Fig. 4. a) The number of days at each site where the minimum air temperature was below 0 °C. b) Estimated number of days at each site where Ψ_{mid} of *P. tremuloides* occurred in different ranges of MPa.

differences in water potentials among individuals across the gradient.

As osmotic adjustments may improve both drought and frost tolerance, we expected that the warmest and coldest sites would exhibit the highest degree of frost tolerance when subjected to a discrete freezing event. Our findings support this hypothesis (Table 2), yet it appears that osmotic adjustment alone is not the mechanism explaining frost tolerance as each site had similar levels of total osmotic adjustment. Seedlings at the coldest site never experienced Ψ_{mid} or VWC conditions as extreme as the other sites, yet increased Ψ_{osm} throughout the season, indicating that other factors such as potentially increasing leaf hardness were impacting seasonal Ψ_{osm} . Furthermore, seedlings at the coldest site exhibited the least negative Ψ_{osm} in September (when the electrolyte leakage test occurred), followed by the intermediate-temperature site, suggesting further investigation of the timing of these adjustments is needed.

We also observed shifts in turgor pressure independent of overall leaf water potentials. Seedlings at the coldest and warmest sites had greater turgor pressure than those at the intermediate-temperature site in August, despite the extreme negative Ψ_{leaf} values at the warmest site (Fig. 3). These results suggest that cell wall and/or membrane characteristics differed between plants growing across the sites. Since the plants had similar amounts of solute concentration in the cells by the end of the season, it could be that the modulus of elasticity of cells from the coldest/warmest sites was higher, which results in greater changes in turgor relative to changes in cell volume. Cell elasticity has been shown to correlate with freezing tolerance in shrubs (Scholz et al., 2012), potentially explaining why electrolyte leakage was not associated with osmotic adjustments across this temperature gradient but were instead indicative of unique responses to abiotic stressors.

Other factors have previously been shown to be important for aspen cold tolerance as well. Welling et al. (2002), using knock-out *P. tremula* x *P. tremuloides* hybrids, determined that both day length and lower temperatures led to cold acclimation, which is similar to our findings were seedlings at the site with the lowest average and minimum temperatures during the study – the coldest site (Table 1, Fig. S2-3) – underwent the least amount of cell damage to a freezing event (Table 2). Our findings also agree with other work on *P. tremula* x *P. tremuloides* hybrids in which electrolyte leakage, along with the production of biochemical compounds associated with frost tolerance, was lower in cold-adapted individuals (Renaut et al., 2005).

We observed the highest above-ground growth efficiency (growth per day of the growing season) at the intermediate-temperature site (Table 2), suggesting that aspen grows most efficiently where populations are local and well adapted to the prevailing conditions, and that shifts away from optimal temperatures result in a shift in strategy away

from higher growth to prioritizing stress and tissue damage avoidance. Allocation of resources to improved frost tolerance at the coldest site and greater osmotic adjustment at the warmest site came at the expense of above-ground growth efficiency. Both water and temperature stress occur in the lowest elevation site, and aspen growth efficiency there was notably low (only 6.5% vs 27.2% and 16.2% at the intermediate and highest sites, respectively), suggesting a response to the environmental conditions by altering carbon allocation to offset abiotic stressors (Table 2; Fig. 4). Indeed, preliminary analyses of growth prioritization at each site suggest that higher temperatures result in trees prioritizing radial stem growth over height growth (Carroll et al., in review). We also found that a shift upslope caused a decline in above-ground growth efficiency as well, potentially due to severe minimum nighttime temperatures, but that reduction was smaller than the combined impact of drought and warming temperatures in the lowest site.

Reduced growth efficiencies and prioritization of stem area over height suggest that aspen are responding to reduced water availability in the soil at the warmest site. Similar work assessing drought tolerance in aspen observed an increased allocation of carbon to structural components, but importantly not an increase in water use efficiency or other behaviors shifts (Anderegg and HilleRisLambers, 2016). They also found that such allocation patterns lead to a decline in basal area growth, suggesting that offsetting the effects of drought with structural carbon is possible but comes at a cost to whole-tree performance.

Recent work by Rodgers et al. (2018) in an old field system in the northeast US also found an interaction between warming and precipitation availability on mortality in seedlings of *Populus grandidentata*, where all warming treatments resulted in increased mortality above ambient conditions. Even with considerable increases in precipitation up to +50%, survival decreased by roughly half with 4 °C of warming. While our temperature increase is not as extreme, we have not increased precipitation and thus we expect similar declines in aspen performance where they are increasingly becoming limited by warming temperatures under similar precipitation levels. This is reflected in our growth efficiency results (Table 2) and is similar to other studies relating drought to reduced historical aspen growth (Chen et al., 2017), forecasts of *Populus* performance declines with future warming (Rehfeldt et al., 2009), and considerable shifts in its optimal habitat in the Rocky Mountains (Gray and Hamann, 2013). Taken together, these results indicate future warming will considerably restrict growth performance of this species.

5. Conclusion

Predictions of future shifts in precipitation and soil moisture in the

western U.S. remain uncertain based in part on the challenges in modeling cloud microphysics and precipitation levels (Kang et al., 2015), while the uncertainty around warming trends in the region is low (Gross et al., 2016). Our experimental approach allowed us to mimic the expected 3 °C of warming by the end of the century. Warming temperatures resulted in decreased available soil moisture and increased days at lower water potentials despite similar ambient precipitation, highlighting the need to incorporate temperature directly into water budgets to generate accurate predictions of future plant performance. While it is generally established that rising temperatures exponentially increase evaporative demand, leaving less water in the soil for plant use, controlled *in situ* work such as our study are important for determining the magnitude and cascading consequences of 3 °C of warming effects on plant function in the western USA. Indeed, seasonal changes in Ψ_{osm} show a tight relationship with water availability, while other factors impacted seasonal adjustments at the cold-limited site. We observed some evidence that shifts in temperature away from the local seed conditions resulted in reductions in growth efficiency to either increase water transport or improve frost tolerance, depending on the environmental constraints. Overall, our results suggest that future aspen performance will be reduced in these regions impacting future aspen distribution and abundance throughout the western U.S.

Author statement

CJWC designed, wrote, edited, and conducted the experiment. PHM, AKK, and TWO contributed to the design, writing, and editing of this manuscript.

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Appendix A. Supplementary data

Supplementary material related to this article can be found, in the online version, at doi:<https://doi.org/10.1016/j.envexpbot.2018.12.014>.

References

- Anderegg, L.D.L., HilleRisLambers, J., 2016. Drought stress limits the geographic ranges of two tree species via different physiological mechanisms. *Glob. Change Biol.* 22, 1029–1045.
- Anderegg, L.D., Anderegg, W.R., Abatzoglou, J., Hausladen, A.M., Berry, J.A., 2013. Drought characteristics' role in widespread aspen forest mortality across Colorado. *USA. Glob. Change Biol.* 19, 1526–1537.
- Anderegg, W.R.L., Flint, A., Huang, C., Flint, L., Berry, J.A., Davis, F.W., Sperry, J.S., Field, C.B., 2015. Tree mortality predicted from drought-induced vascular damage. *Nat. Geosci.* 8, 367.
- Bartlett, M.K., Klein, T., Jansen, S., Choat, B., Sack, L., 2016. The correlations and sequence of plant stomatal, hydraulic, and wilting responses to drought. *Proc. Natl. Acad. Sci.* 113, 13098–13103.
- Bates, D., Mäechler, M., Bolker, B., Walker, S., 2015. Fitting linear mixed-effects models using lme4. *J. Stat. Softw.* 67 (1), 1–48.
- Boyer, J.S., 1970. Differing sensitivity of photosynthesis to low leaf water potentials in corn and soybean. *Plant Physiol.* 46, 236–239.
- Bretfeld, M., Franklin, S.B., Peet, R.K., 2016. A multiple-scale assessment of long-term aspen persistence and elevational range shifts in the Colorado Front Range. *Ecol. Monogr.* 86, 244–260.
- Carroll, C.J., Knapp, A.K., Martin, P.H., 2017. Dominant tree species of the Colorado Rockies have divergent physiological and morphological responses to warming. *For. Ecol. Manag.* 402, 234–240.
- Carroll, C.J.W., P.H. Martin, and A.K. Knapp. In review. Growth and survivorship of the tree species respond positively to hotter temperatures in Rocky Mountain ecosystems.
- Chen, L., Huang, J.-G., Alam, S.A., Zhai, L., Dawson, A., Stadt, K.J., Comeau, P.G., 2017. Drought causes reduced growth of trembling aspen in western Canada. *Glob. Change Biol.* 23, 2887–2902.
- Choat, B., Jansen, S., Brodribb, T.J., Cochard, H., Delzon, S., Bhaskar, R., Bucci, S.J., Feild, T.S., Gleason, S.M., Hacke, U.G., et al., 2012. Global convergence in the vulnerability of forests to drought. *Nature* 491, 752–755.
- Dudley, M.M., Burns, K.S., Jacobi, W.R., 2015. Aspen mortality in the Colorado and southern Wyoming Rocky Mountains: extent, severity, and causal factors. *For. Ecol. Manag.* 353, 240–259.
- Gray, L.K., Hamann, A., 2013. Tracking suitable habitat for tree populations under climate change in western North America. *Clim. Change* 117, 289–303.
- Gross, J.E., Tercek, M., Guay, K., Talbert, M., Chang, T., Rodman, A., Thoma, D., Jantz, P., Morissette, J.T., 2016. Analyses of historical and projected climates to support climate adaptation in the Northern Rocky Mountains. *Climate Change in Wildlands*. Island Press, Washington, DC, pp. 55–77.
- Kang, I.-S., Yang, Y.-M., Tao, W.-K., 2015. GCMs with implicit and explicit representation of cloud microphysics for simulation of extreme precipitation frequency. *Clim. Dyn.* 45, 325–335.
- Lenth, R.V., 2016. Least-squares means: the R Package lsmeans. *J. Stat. Softw.* 69 (1), 1–33.
- McDowell, N., Pockman, W.T., Allen, C.D., Breshears, D.D., Cobb, N., Kolb, T., Plaut, J., Sperry, J., West, A., Williams, D.G., et al., 2008. Mechanisms of plant survival and mortality during drought: why do some plants survive while others succumb to drought? *New Phytol.* 178, 719–739.
- McGuire, C.R., Nufio, C.R., Bowers, M.D., Guralnick, R.P., 2012. Elevation-dependent temperature trends in the rocky mountain front range: changes over a 56- and 20-Year record. *PLoS One* 7, e44370.
- Michaelian, M., Hogg, E.H., Hall, R.J., Arseneault, E., 2011. Massive mortality of aspen following severe drought along the southern edge of the Canadian boreal forest. *Glob. Change Biol.* 17, 2084–2094.
- Murphy, L., 2015. Tools for Maximum Likelihood Estimation of Parameters of Scientific Models.
- Parker, J., Patton, R.L., 1975. Effects of drought and defoliation on some metabolites in roots of black oak seedlings. *Can. J. For. Res.* 5, 457–463.
- Pramsohler, M., Neuner, G., 2013. Dehydration and osmotic adjustment in apple stem tissue during winter as it relates to the frost resistance of buds. *Tree Physiol.* 33, 807–816.
- PRISM Climate Group, 2004. Oregon State University. created 4 Feb. <http://prism.oregonstate.edu>.
- Puigdefabregas, J., Pugnaire, F.I., 1999. Plant survival in arid environments. In: Pugnaire, F.I., Valladares, F. (Eds.), *Handbook of Functional Plant Ecology*. Marcel Dekker, pp. 381–405.
- Rehfeldt, G.E., Ferguson, D.E., Crookston, N.L., 2009. Aspen, climate, and sudden decline in western USA. *For. Ecol. Manag.* 258, 2353–2364.
- Renaut, J., Hoffmann, L., Hausman, J.-F., 2005. Biochemical and physiological mechanisms related to cold acclimation and enhanced freezing tolerance in poplar plantlets. *Physiol. Plant.* 125, 82–94.
- Rodgers, V.L., Smith, N.G., Hoepfner, S.S., Dukes, J.S., 2018. Warming increases the sensitivity of seedling growth capacity to rainfall in six temperate deciduous tree species. *AoB Plants* 10 ply003.
- Sánchez Viveros, G., gonzalez-mendoza, D., Alarcon, A., Ferrera-Cerrato, R., 2010. Copper effects on photosynthetic activity and membrane leakage of *Azolla filiculoides* and *A. caroliniana*. *Int. J. Agric. Biol.* 12, 365–368.
- Scholz, F.G., Bucci, S.J., Arias, N., Meinzer, F.C., Goldstein, G., 2012. Osmotic and elastic adjustments in cold desert shrubs differing in rooting depth: coping with drought and subzero temperatures. *Oecologia* 170, 885–897.
- Tai, X., Mackay, D.S., Anderegg, W.R.L., Sperry, J.S., Brooks, P.D., 2017. Plant hydraulics improves and topography mediates prediction of aspen mortality in southwestern USA. *New Phytol.* 213, 113–127.
- Webster, D.E., Ebdon, J.S., 2005. Effects of nitrogen and potassium fertilization on perennial ryegrass cold tolerance during deacclimation in late winter and early spring. *HortScience* 40, 842–849.
- Welling, A., Moritz, T., Palva, E.T., Junttila, O., 2002. Independent activation of cold acclimation by low temperature and short photoperiod in hybrid aspen. *Plant Physiol.* 129, 1633–1641.
- Wickham, H., Francois, R., Henry, L., Müller, K., Studio, R., 2017. A Grammar of Data Manipulation. <https://github.com/tidyverse/dplyr>. <http://dplyr.tidyverse.org>.
- Wisniewski, M., Bassett, C., Gusta, L.V., 2003. An overview of cold hardiness in woody plants: seeing the forest through the trees. *HortScience* 38, 952–959.
- Worrall, J.J., Marchetti, S.B., Egeland, L., Mask, R.A., Eager, T., Howell, B., 2010. Effects and etiology of sudden aspen decline in southwestern Colorado. *USA. For. Ecol. Manag.* 260, 638–648.
- Wu, Z., Dijkstra, P., Koch, G.W., Peñuelas, J., Hungate, B.A., 2011. Responses of terrestrial ecosystems to temperature and precipitation change: a meta-analysis of experimental manipulation. *Glob. Change Biol.* 17, 927–942.
- Zwieniecki, M.A., Secchi, F., 2015. Threats to xylem hydraulic function of trees under “new climate normal” conditions. *Plant Cell Environ.* 38, 1713–1724.